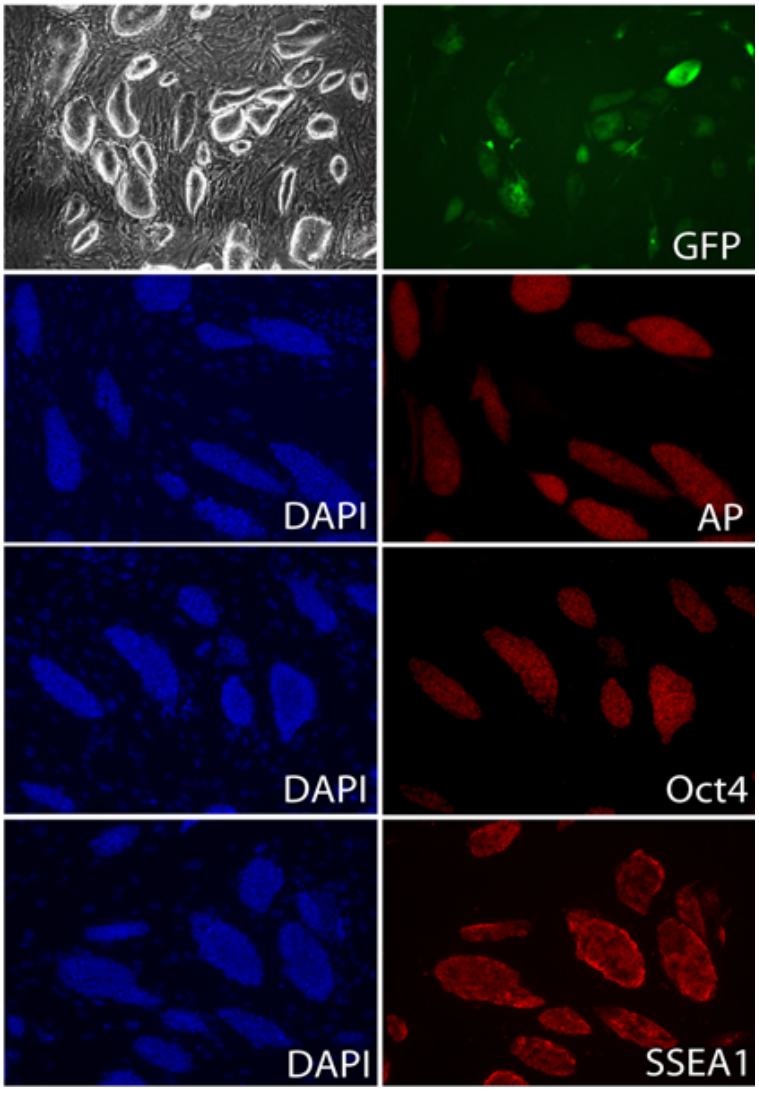
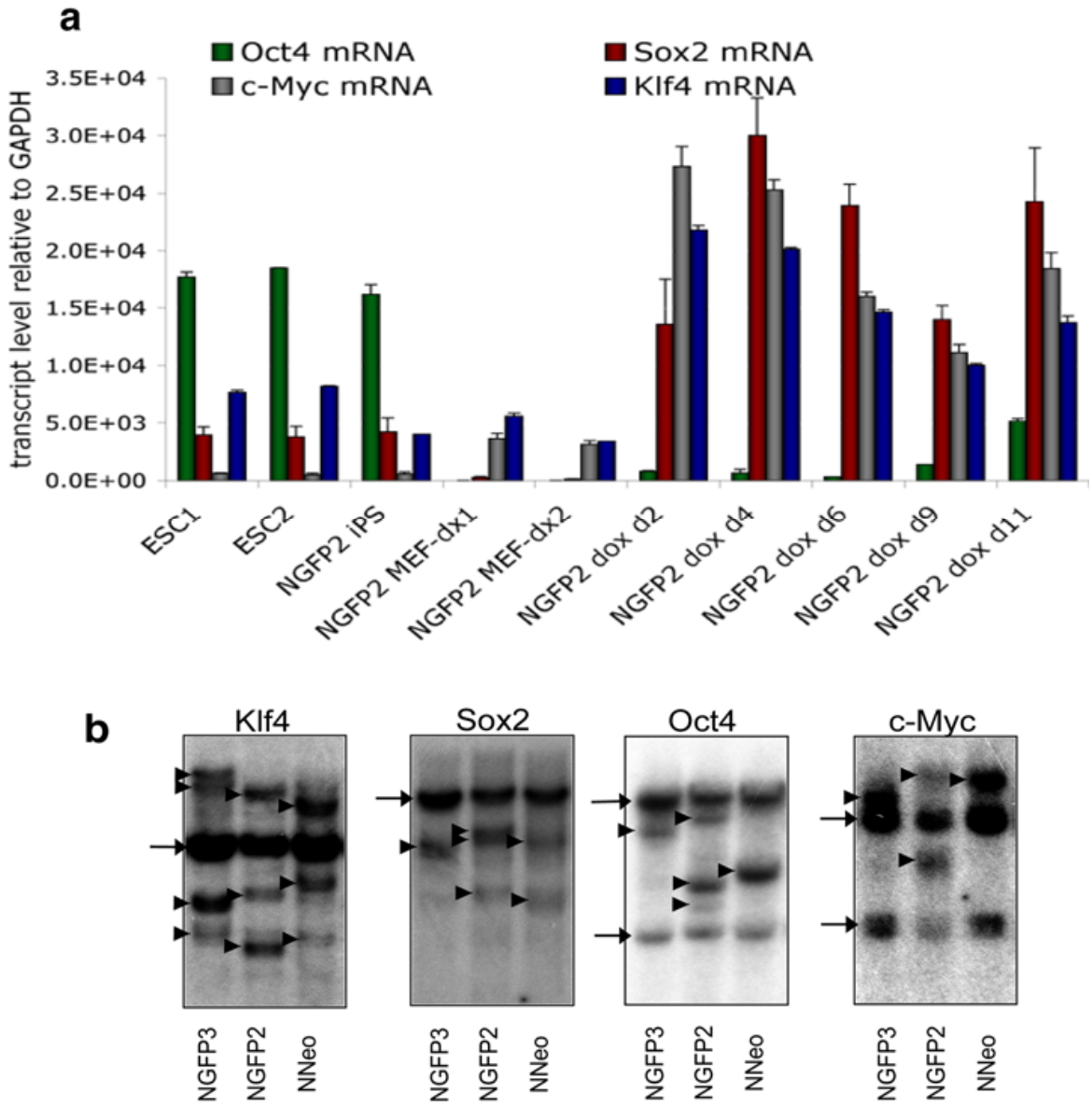
Supplementary Figure 1



Supplementary Figure 1

Pluripotence gene reactivation in MEF-derived secondary iPS cells
Fully reprogrammed NGFP2 secondary MEFs reactivated the endogenous Nanog locus, express Oct4, AP, and SSEA1, and could be maintained in the absence of dox.

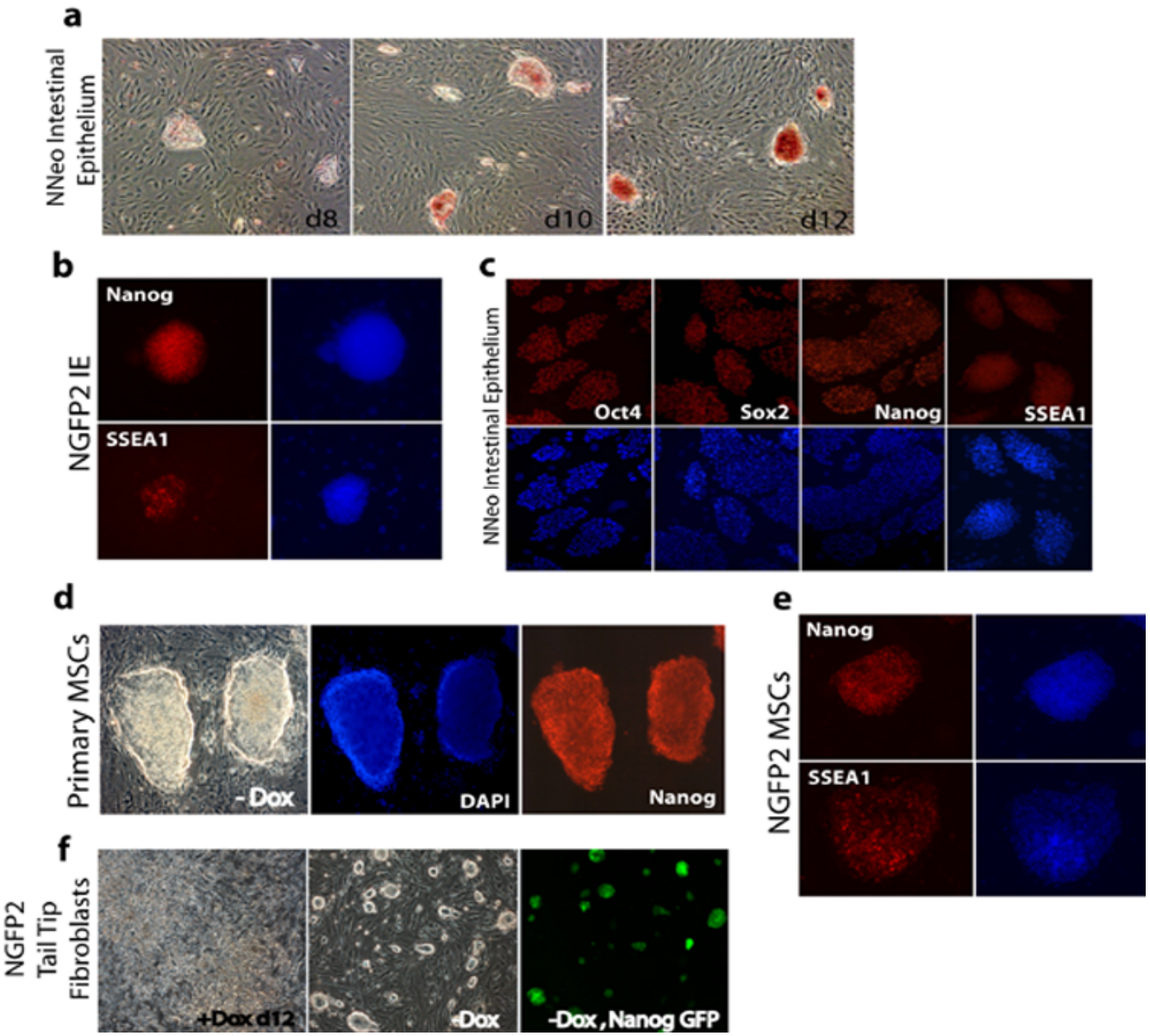
Supplementary Figure 2



Supplementary Figure 2

Transgene copy number and activation during reprogramming
(a) qRT-PCR analysis of endogenous Oct4, Sox2, Klf4, and c-Myc transcripts in NGFP2 MEFs during the time course of reprogramming in response to dox treatment. Also shown are expression levels in two ES cell RNA preparations (V6.5 line) and the NGFP2 iPS cell line. **(b)** Southern analysis of secondary iPS lines NGFP3, NGFP2, and NNeo with Klf4, c-Myc, Sox2, and Oct4 cDNA probes. Endogenous bands marked with arrow, proviral insertions marked with arrowhead, with the exception of Oct4 in the NNeo line, which is a transgene targeted to the collagen I locus.

Supplementary Figure 3

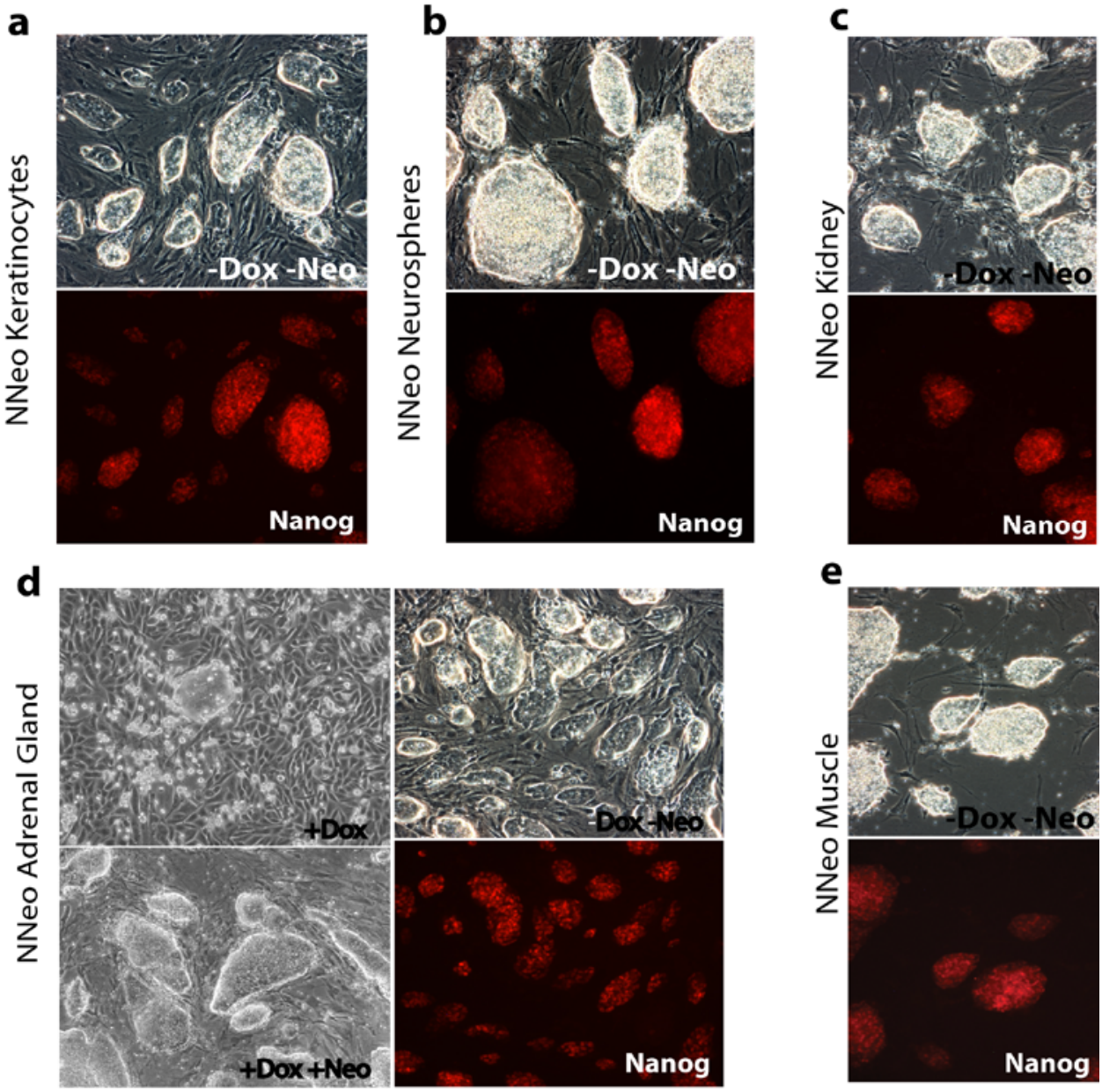


Supplementary Figure 3

Pluripotency gene reactivation in tissue-derived iPS cells

(a) Secondary intestinal epithelium isolated from NNeo chimeras and cultured in the presence of dox for 8, 10, or 12 days and stained for alkaline phosphatase activity. **(b)** iPS cells derived from NGFP2 secondary intestinal epithelium express endogenous Nanog and SSEA1. **(c)** NNeo secondary intestinal epithelial cells became dox-independent iPS cells after infection with additional Sox2 and Klf4 viruses. Immunofluorescence analysis (red, top row) revealed expression of Oct4, Sox2, Nanog, and SSEA1 in fully reprogrammed cells (blue, bottom row- DAPI DNA stain). **(d)** Primary mesenchymal stem cells harboring the reverse tetracycline transactivator at the *Rosa 26* locus and the *Oct4* coding sequence under control of the Tet-operator[16] were infected with viruses encoding *Sox2*, *c-Myc*, and *Klf4*. Addition of dox to the infected MSCs resulted in fully reprogrammed, dox-independent iPS cells that express endogenous Nanog protein (immunofluorescence). **(e)** iPS cells derived from NGFP2 secondary mesenchymal stem cells express endogenous Nanog and SSEA1. **(f)** Secondary NGFP2 tail tip fibroblasts successfully reprogrammed into dox-independent, GFP+ iPS cells.

Supplementary Figure 4



Supplementary Figure 4

Pluripotency gene reactivation in tissue-derive NNeo secondary iPS cells
Successful reprogramming of cell cultures derived from the keratinocytes (**a**), neurospheres (**b**), kidney (**c**), adrenal gland **(d)**, and muscle **(e)** of NNeo secondary chimeras determined by dox independence, neomycin resistance, and Nanog expression (red, immunofluorescence).